Sympathetic Activity and Body Mass Index Contribute to Blood Pressure Levels

Kazuko MASUO, Hiroshi MIKAMI*, Mikiko ITOH*, Toshio OGIHARA, and Michael L. TUCK**

The purpose of this study was to clarify the relationships between obesity (BMI) and BP levels, leptin levels, sympathetic activity, and insulin sensitivity in a Japanese male population. In 912 young, non-diabetic, Japanese men with a wide range of BMI (16.5-33.6 kg/m²), blood pressure (BP), fasting plasma norepinephrine (NE), insulin and leptin levels were measured after an overnight fast. The cohort consisted of 603 normotensive and 309 hypertensive subjects. The study was carried out using a cross-sectional design. When the subjects were subdivided by tertile in relation to BMI, the 101 subjects in the heaviest group (BMI > 27.9 kg/m²) had a significantly higher systolic BP (p<0.05) and pulse rate (p<0.05) as well as higher NE (p<0.01), insulin (p<0.01), and leptin (p<0.01) levels than 86 subjects in the leanest group (BMI < 22.2 kg/m²). In the whole cohort, BMI correlated with mean BP (p<0.01), plasma NE (p<0.05), insulin (p<0.001) and leptin (p<0.001). The mean BP correlated with BMI (p<0.001), plasma NE (p<0.01), insulin (p<0.01) and leptin (p<0.05). Plasma leptin levels correlated with fasting plasma insulin levels (p<0.05), but not with plasma NE levels (NS). As analyzed by multiple regression analysis, only plasma NE (p<0.05) and BMI (p<0.001), but not plasma insulin levels, were significant, independent predictors of BP levels (r²=0.125, F=10.51, p=0.0001). These results suggest that obesity (BMI) and heightened sympathetic nervous system activity contribute to BP elevation (hypertension).

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Key Words: body mass index, obesity, blood pressure, sympathetic activity, leptin, insulin

Introduction

It is well known that obesity is often associated with elevated blood pressure (1), and that obesity is an independent direct risk factor for cardiovascular disease (2) as well as an indirect risk factor because of its links to diabetes, hypertension, and hyperlipidemia (3). Sympathetic hyperactivity (4-10) and hyperinsulinemia (11-18) appear to be to some extent responsible for this tendency towards hypertension or blood pressure elevation in obese subjects. Regarding the heightened sympathetic nervous system activity in the obese, 1) we have previously reported that the higher levels of plasma norepinephrine (NE), insulin and leptin can be observed in obese subjects regardless of BP status in weight-reduction studies (10). 2) In addition, Esler et al. (9) have reported the use of spillover methods for measurements of whole-body and regional sympathetic activity which show that the increases in renal sympathetic activity in obesity may be necessary to the development of hypertension in obese individuals. However, 3) Young and Macdonald recently analyzed 43 studies addressing the sympathetic activity in obesity. In their review, they note that 19 studies found no difference in sympathetic activity (plasma NE levels) between lean and obese subjects, 14 studies found NE to
be lower in obese than lean subjects, and 11 found higher NE in obese compared to lean subjects (19). They suggest that this diversity of findings for plasma NE might be dependent on factors such as age, the number of subjects, ethnicity, diet, BP level, gender, glucose tolerance and physical activity.

Since leptin was discovered as a hormone (20) produced by an adipocyte-specific obese gene, it has been reported that leptin contributes to the regulation of energy balance by informing the hypothalamus of the amount of adipose tissue in the body (21, 22). In addition, it has been reported that leptin may have multiple actions on sympathetic nervous activity, insulin sensitivity, obesity-related hypertension, and cardiovascular function. However, whether leptin contributes to the increased arterial pressure in obese humans (obesity-related hypertension), and the precise relations between leptin and sympathetic activity or insulin sensitivity have not yet been clarified.

The goal of the present study was to clarify the relationships between BMI (obesity) and variables such as blood pressure and fasting plasma norepinephrine levels as an index of sympathetic nervous system activity as well as leptin and insulin levels in a young, non-diabetic, Japanese male population.

**Subjects**

A cohort of 1,497 Japanese men working in a factory in Osaka, Japan, were studied on an annual basis. Excluded were 157 women, 121 older (>50 years) subjects, 47 with diabetes mellitus (HbA1c > 6.0%), 13 with heart disease, and 47 with renal dysfunction (creatinine > 1.5 mg/dl). Additional exclusions were 109 subjects who were taking medications other than antihypertensive drugs, 98 subjects who had experienced significant changes in BMI (>10%) in the year before the study period began, and 95 subjects whose informed consents were not obtained. In addition, 102 subjects had two or three of the exclusion criteria and were therefore not included in the study. Consequently, after the enrolled subjects were subdivided by tertile according to BMI, 101 lean subjects were included in the leanest group (BMI < 22.2 kg/m²), 725 middle-weight subjects in the middle group (BMI 22.2-27.9 kg/m²), and 86 obese subjects in the heaviest group (BMI ≥ 27.9 kg/m²). One hundred seventy-four borderline hypertensive subjects (BP 140-159/90-94 mmHg) were untreated, and 135 established hypertensive subjects (BP ≥ 160/95 mmHg) were untreated or stopped their medications for hypertension at least 1 week prior to the measurements. Either calcium channel blockers, ACE-inhibitors, or other antihypertensive agents were used to treat 121 (90%) subjects with established hypertension. Informed consent was obtained from each subject as approved by the Ethics Committee of Osaka University Medical School.

**Measurements**

After a 12-h overnight fast, blood pressure (BP), pulse rate, body mass index (BMI), and % fat accumulation were determined in the subjects. After the subjects had rested for 30 min in the supine position in a quiet room, venous blood was taken for blood glucose, plasma norepinephrine (NE), insulin and leptin levels. Supine BP was measured three times and then averaged. The percent fat accumulation was determined with impedance measurements (BF102, Tanita, Tokyo, Japan). BP and pulse rate were measured with an automated sphygmanometer (TM-2711 or TM-2713, A&D, Tokyo, Japan), which was standardized against a mercury sphygmanometer. Plasma NE was measured by the fluorometric method after separation by high-performance liquid chromatography. In this assay, the intra-assay CV was 2.1%, the inter-assay CV 3.6%, and the sensitivity 0.01 to 20 ng/ml. Plasma immunoreactive insulin was measured by a standard radioimmunoassay method (insulin RIA BEAD II, Dianabott). The intra-assay CV was 1.9%, the inter-assay CV 2.2%, and sensitivity 0.75 to 300 μU/ml. Plasma leptin was measured by radioimmunoassay (human leptin RIA kit, Linco). The intra-assay CV was 5.0%, the inter-assay CV 4.5%, and the sensitivity 0.5 to 100 ng/ml. Blood glucose was measured by an autoanalyzer (Hitachi-7050).

**Statistical Analysis**

Values are shown as mean ± SD. The values obtained in the groups according to BMI were compared with those for the lean (the leanest) group by one-way analysis of variance using Dunnett's test for subsequent comparisons. Pearson’s correlation coefficient was calculated by linear regression. Multiple linear regression analyses were used to examine independent relations, using either the mean BP, systolic BP, or diastolic BP levels as the dependent variable, and BMI (or % fat accumulation) as well as fasting plasma norepinephrine, insulin and leptin levels as independent variables. The statistical analyses to compare the prevalence of hypertensive subjects in each group were performed by the $\chi^2$ (chi square) test. Values of $p < 0.05$ were considered to be statistically significant.

**Results**

Table 1 shows the demographic values of the subjects: mean age, systolic and diastolic levels of BP, pulse rate, mean BMI, mean %fat accumulation, and mean fasting blood glucose, plasma insulin, NE and leptin levels in the three groups subdivided by the tertile of BMI levels (16.5–33.6 kg/m²); specifically, there were 101 subjects in the lean group (the leanest group), 725 in the middle-weight group (the middle group) and 86 in the obese...
The mean age and range in age (22-50 years) were strictly matched among the three study groups. The obese group had significantly higher levels of BP, pulse rate, plasma NE, insulin and leptin than the lean group, as did the middle-weight group. The prevalence of hypertensive (> 140/90 mmHg) subjects in the obese group (48%) was greater than that in the lean group (18%) (Table 1).

Table 1. The Demographic Values of the 3 Study Groups Subdivided into the Tertiles of Body Mass Index

<table>
<thead>
<tr>
<th></th>
<th>Lean subjects</th>
<th>Middle-weight subjects</th>
<th>Obese subjects</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number</td>
<td>101</td>
<td>725</td>
<td>86</td>
</tr>
<tr>
<td>Percent of hypertensives</td>
<td>18% (18)</td>
<td>34% * (250)</td>
<td>48% <em>.</em> (41)</td>
</tr>
<tr>
<td>Mean age (years)</td>
<td>43±4</td>
<td>43±2</td>
<td>45±3</td>
</tr>
<tr>
<td>Mean BMI (kg/m²)</td>
<td>20.1±1.7 #</td>
<td>22.5±2.2 *</td>
<td>30.0±1.8 **.#</td>
</tr>
<tr>
<td>Range of BMI (kg/m²)</td>
<td>16.5≤&lt;22.2</td>
<td>22.2-27.9</td>
<td>27.9≤&lt;33.6</td>
</tr>
<tr>
<td>%Fat accumulation (%)</td>
<td>5.3±2.3 ###</td>
<td>21.8±1.3 **</td>
<td>33.5±5.4 **.#</td>
</tr>
<tr>
<td>Blood pressure (mmHg)</td>
<td>128±12#/77±9</td>
<td>133±5*/79±7</td>
<td>139±8*/84±6</td>
</tr>
<tr>
<td>Pulse rate (beats/min)</td>
<td>71±4#</td>
<td>74±5*</td>
<td>77±4**</td>
</tr>
<tr>
<td>Plasma norepinephrine (pg/ml)</td>
<td>127±58#</td>
<td>178±32*</td>
<td>297±98**.#</td>
</tr>
<tr>
<td>Plasma insulin (µU/ml)</td>
<td>4.9±3.4 #</td>
<td>9.1±3.1 *</td>
<td>21.9±11.8 **.#</td>
</tr>
<tr>
<td>Plasma leptin (ng/ml)</td>
<td>2.9±0.4 #</td>
<td>3.9±0.5 *</td>
<td>9.4±2.2**</td>
</tr>
</tbody>
</table>

* p<0.05, ** p<0.01 compared with values in the lean subjects. # p<0.05, ## p<0.01 compared with values in the middle-weight subjects. BMI: body mass index.

Table 2. Pearson’s Correlation Coefficients between Body Mass Index, Blood Pressure Levels and Other Variables in the Whole Study Cohort

<table>
<thead>
<tr>
<th></th>
<th>Age</th>
<th>BMI</th>
<th>Mean BP</th>
<th>Systolic BP</th>
<th>Diastolic BP</th>
</tr>
</thead>
<tbody>
<tr>
<td>BMI</td>
<td>0.044</td>
<td>0.285 ***</td>
<td>0.294 ***</td>
<td>0.255 ***</td>
<td></td>
</tr>
<tr>
<td>%Fat accumulation</td>
<td>0.037</td>
<td>0.684 ***</td>
<td>0.120*</td>
<td>0.144*</td>
<td>0.092</td>
</tr>
<tr>
<td>Fat accumulation</td>
<td>0.041</td>
<td>0.853 ***</td>
<td>0.173 **</td>
<td>0.200 ***</td>
<td>0.138 *</td>
</tr>
<tr>
<td>Mean BP</td>
<td>0.059</td>
<td>0.285 ***</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Systolic BP</td>
<td>0.069</td>
<td>0.294 ***</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Diastolic BP</td>
<td>0.050</td>
<td>0.255 ***</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Plasma norepinephrine</td>
<td>0.069</td>
<td>0.107 *</td>
<td>0.168 **</td>
<td>0.189 ***</td>
<td>0.138 *</td>
</tr>
<tr>
<td>Fasting plasma insulin</td>
<td>0.058</td>
<td>0.242 ***</td>
<td>0.138 **</td>
<td>0.145 **</td>
<td>0.042</td>
</tr>
<tr>
<td>Fasting plasma leptin</td>
<td>0.039</td>
<td>0.505 ***</td>
<td>0.132 *</td>
<td>0.118 *</td>
<td>0.123 *</td>
</tr>
</tbody>
</table>

* p<0.05, ** p<0.01, *** p<0.001, BMI: body mass index, BP: blood pressure.

We analyzed systolic BP, diastolic BP and mean BP, respectively, as independent factors in association with BMI and plasma NE, insulin and leptin levels as determinant factors by multiple regression analysis (Table 3). BMI and plasma NE levels were significant determinant factors in relation to mean BP as well as to systolic BP and diastolic BP when analyzed by multiple regression analyses. In conclusion, the determinant factors for mean BP were BMI and plasma NE levels, but not insulin or leptin levels, as analyzed by multiple regression analyses. When using the %fat accumulation in place of BMI, similar results were obtained in the multiple regression analyses.

In lean subjects, the mean BP levels correlated only with plasma NE levels, but did not with plasma insulin and leptin levels (Fig. 2). On the other hand, in obese subjects, mean BP levels correlated with plasma NE, insulin and leptin (Fig. 3). Plasma leptin levels did not correlate either with plasma NE levels or fasting plasma insulin levels in lean subjects, but did so with those in obese subjects (not shown).
Discussion

The primary findings of the present study are as follows: 1) systolic BP, pulse rate and plasma NE, insulin, and leptin levels in the obese group of the tertile were significantly higher than those in lean group, as has been previously reported (23-27); 2) BP levels correlated with plasma NE, insulin, and leptin levels in the whole cohort in Pearson's correlation analysis, as has been previously reported (28-30). However, the novel finding of the present study is that in multiple regression analyses only BMI and plasma NE levels, but not insulin or leptin levels, are predictors of BP levels in a non-diabetic, young, but with a wide range of BMI, male population.

In obese subjects in the present study, mean BP correlated with plasma NE, insulin and leptin levels, although the mean BP in lean subjects correlated only with plasma NE levels, but not with fasting plasma insulin nor leptin levels. It should be noted that sympathetic activity, leptin, insulin, BP levels, pulse rates and BMI were measured at the same time in a large number of subjects (912) with a wide range of BMI even in this cross-sectional study. These results suggest that BP levels are primarily dependent on sympathetic activity and obesity (BMI). Furthermore, higher level of plasma leptin appears to be a trigger for the stimulation of sympathetic activity in obese subjects.

The present study considered only male subjects because it is well known that there are gender differences in plasma leptin levels. Furthermore, it is well documented that age-related factors can explain some of the differences in BP, sympathetic activity and insulin (3I) and leptin levels. However, this study was done only in young men distributed in a narrow age-range (22-50 years), and no differences were noted in the mean age and standard

Table 3. Multiple Regression Analyses for Mean Blood Pressure, Systolic Blood Pressure and Diastolic Blood Pressure

<table>
<thead>
<tr>
<th>Variables</th>
<th>Mean BP</th>
<th>Systolic BP</th>
<th>Diastolic BP</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>F value</td>
<td>p value</td>
<td>F value</td>
</tr>
<tr>
<td>Body mass index</td>
<td>22.31</td>
<td>0.0001</td>
<td>22.69</td>
</tr>
<tr>
<td>Plasma norepinephrine</td>
<td>8.28</td>
<td>0.0042</td>
<td>11.11</td>
</tr>
<tr>
<td>Plasma insulin</td>
<td>1.47</td>
<td>0.2260</td>
<td>0.19</td>
</tr>
<tr>
<td>Plasma leptin</td>
<td>0.57</td>
<td>0.4502</td>
<td>0.40</td>
</tr>
</tbody>
</table>

\[ r^2=0.125, \quad F=10.51, \quad p=0.0001 \]
\[ r^2=0.144, \quad F=12.86, \quad p=0.0001 \]
\[ r^2=0.093, \quad F=7.51, \quad p=0.0001 \]

Mean BP: mean blood pressure, Systolic BP: systolic blood pressure, Diastolic BP: diastolic blood pressure.
deviation in age among the three study groups. Therefore, the differences in BP, plasma NE, insulin and leptin levels among the three study groups appear to depend on BMI rather than age.

Thus, obese subjects had higher BP, pulse rate, and plasma NE, insulin and leptin levels than lean subjects, suggesting that heightened sympathetic nervous system activity such as a faster pulse rate, and elevated levels of plasma NE, insulin and leptin might be related to obesity-related BP elevations. Recent animal studies indicate that leptin may influence autonomic and cardiovascular function (8, 27, 32, 33). In addition, elevated leptin levels have been suggested to contribute to blood pressure increases in obesity through an activation of the sympathetic nervous system in animal models (7, 8, 27). Acute intravenous infusion of leptin in rats increases sympathetic nerve activity to the kidney, hindlimb and adrenal grand (8, 27). In addition, Hall and colleagues (34) have recently demonstrated that a chronic infusion of leptin elevates arterial pressure and heart rate in unanesthetized Sprague-Dawley rats, suggesting a possible role of leptin in obesity-induced hypertension in rodents. In addition, the arterial pressure, heart rate and sympathetic nervous system responses to physiological increases in circulation leptin levels appear to be slow in onset (34). In humans, Collins et al. have reported that leptin increases norepinephrine turnover in brown adipose tissue (35). Nar- kiewicz et al. have also reported based on a cross-sectional study of 60 men with untreated mild hypertension that ambulatory awake heart rates and night-time heart rates are faster than expected in those with higher plasma leptin levels when patients are divided into tertiles of BMI-adjusted plasma leptin levels (23). Their results also have implications for improving our understanding of interactions between leptin and sympathetic activity in hypertensive humans, as they demonstrate the close relationships between leptin, sympathetic activity and BP in animal and human studies. However, the mechanisms by which increased circulating levels of leptin chronically elevate arterial pressure and heart rate are not entirely clear.

Fig. 2. Relationships of plasma norepinephrine levels (left panel, r=0.22, p<0.05), fasting plasma insulin levels (middle panel, NS) and plasma leptin levels (right panel, NS) with mean blood pressure levels in lean subjects.

Fig. 3. Relationships of plasma norepinephrine levels (left panel, r=0.29, p<0.05), fasting plasma insulin levels (middle panel, r=0.24, p<0.05), and plasma leptin levels (right panel, r=0.31, p<0.05) with mean blood pressure levels in obese subjects.
Therefore, in the present study, we measured both plasma NE levels and pulse rate as direct and indirect indices, respectively, of sympathetic activity, as well as plasma leptin and BP levels in humans with a wide range of BMI. We found that obese subjects had significantly higher BP, pulse rate, and plasma NE, insulin and leptin levels than lean subjects, suggesting that obese subjects with higher levels of plasma leptin might be related to heightened sympathetic activity and BP elevation. However, additional studies are required to determine the contribution of sympathetic mechanisms in mediating the effects of leptin on blood pressure because of the cross-sectional design of the present study.

Plasma NE levels and BMI correlated significantly with BP levels in the whole cohort in multiple regression analysis, whereas plasma leptin and insulin levels did not, suggesting that heightened sympathetic activity, but not hyperinsulinemia or hyperleptinemia, is related with obesity appears to play an important role in obesity-related hypertension. In addition, BMI correlated with BP levels, suggesting another possibility that obesity itself is directly related to hypertension (BP elevation). Poehlman et al. (25) and Christin et al. (36) have demonstrated that whole-body NE spillover has lower clearance rates in subjects with more central adiposity. In addition, Grassi et al. (5) previously reported using microneurography at the peroneal nerve that baseline muscle sympathetic nerve activity in obese normotensive subjects is twice that of lean control subjects. Esler and colleagues (6, 7) have also demonstrated that mean cardiac NE spillover is 46% lower in obese normotensives than in lean normotensives, although they showed that renal NE spillover correlated with BMI using regional NE kinetics method. We have recently reported that elevated sympathetic activity might be strongly related to obesity-related BP elevations in both normotensive and hypertensive men (10). These reports provide direct, unequivocal evidence that human obesity is associated with a markedly sympathetic activation. In the present study, obese subjects had significantly higher levels of plasma NE and BP than lean subjects, demonstrating that obesity is associated with both hypertension (BP elevation) and elevated sympathetic activity, which are probably triggered by elevated leptin levels.

It is well documented that obesity or fat accumulation related to hyperinsulinemia is an important factor in the development of insulin resistance. Leptin itself has been reported to decrease insulin levels through neuropeptide-Y suppression (37). On the other hand, several investigations have demonstrated that long-term effects of insulin on leptin production can be observed both in vitro (38, 39), and also that during 2 h of euglycemic hyperinsulinemic clamp, plasma leptin concentrations remain constant in both lean and obese subjects (40). Hildebrandt and Hall et al. (41) have recently reported that chronic insulin administration is unable to increase blood pressure in dogs given high doses of insulin for 28 days under conditions of euglycemia. However, Hall and colleagues have been able to induce hypertension through the chronic administration of insulin and dextrose in rats (42). To determine whether chronic hyperinsulinemia can elevate blood pressure, Meehan et al. (15) administered insulin via osmotic minipumps to normal rats for 10 days, finding that the blood pressure response is exaggerated by exogenous insulin administration, preventing a sympathetic blockade. Their results suggest that hyperinsulinemia can contribute to a sympathetically mediated rise in blood pressure in normal rats. Interestingly, the infusion of insulin in subjects with established insulin resistance such as obese subjects has been shown to produce vasoconstriction rather than vasodilation (40, 43). Thus, data regarding the effects of chronic exogenous administration of insulin and resultant hyperinsulinemia on blood pressure are controversial. In the present study, however, plasma insulin levels, but not plasma NE, correlated significantly with plasma leptin levels in the whole cohort, suggesting that plasma insulin levels regulate plasma leptin levels in a young, male population.

In the present study, multiple regression analyses failed to show a significant relationship between insulin and leptin levels, and BP in the whole cohort. This failure may have been due to the influence of the 121 treated hypertensive patients, because we have recently reported that long-term treatments of hypertension with ACE-inhibitors and calcium channel blockers have some influence on plasma NE, insulin and leptin levels (44). In addition, some kinds of antihypertensive agents are known to exert a depressor effect for more than 1 week after withholding medication. However, as we analyzed only normotensive subjects not under the influence any antihypertensive agent, we found no significant relationship between insulin, leptin, and BP levels in our multiple regression analyses. In addition, this study was cross-sectional in nature and hence could not determine the temporal order of events, and particularly whether an increase in leptin, norepinephrine and insulin preceded the BP elevation or hypertension. We must admit that longitudinal data concerning the temporal order of events would be valuable, particularly for young subjects with weight gain-induced BP elevation (obesity induced-hypertension).

The usage of plasma NE levels as an index of sympathetic nerve activity may be regarded as a limitation of the present study. Grassi and Esler (45) have reported that NE spillover and muscle sympathetic nerve activity is a better index of sympathetic nerve activity than plasma NE concentrations. However, they have also reported that the spillover of NE and muscle sympathetic nerve activity is different in each organ. In addition, simple measurement of catecholamine levels appear to be a less than ideal marker of sympathetic nervous system activity. Upon release, NE rapidly undergoes re-uptake so that
only a small fraction reaches the peripheral circulation. Studies sampling venous blood from the forearm to determine NE levels have revealed that half of the NE is derived from the forearm itself and only half from the rest of the body. However, plasma and urine NE measurements are the most commonly used indicators of sympathetic nervous system activity in clinical and epidemiological studies, and these measurements might still be useful, especially with a large number of subjects such as that in the present study.

In conclusion, the present study has demonstrated that marked sympathetic hyperactivity and BMI are associated with obesity-related BP elevation (obesity-related-hypertension). These results suggest that obesity and sympathetic hyperactivity contribute to BP elevation (hypertension), and that hyperleptinemia associated with hyperinsulinemia might be a trigger for stimulated sympathetic tone, especially in obese subjects. Further studies are needed, however, to determine the more precise role of sympathetic activity, insulin sensitivity and leptin in BP regulation in obesity.

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